

The University of Chicago

CELL MEMBRANE IN VEGETABLES DURING COOKING

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS AND
HOUSEHOLD ADMINISTRATION

1939

By

JEAN I. SIMPSON

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CHEMICAL AND HISTOLOGICAL STUDIES OF THE DIS- INTEGRATION OF CELL-MEMBRANE MATERIALS IN VEGETABLES DURING COOKING

The purpose of this study has been to observe some of the chemical and structural changes which take place in vegetables during cooking and which are in all probability related to the softening known to occur. Inasmuch as it is the cell wall which serves to give rigidity (texture) to the plant, Stiles (1937) and Anderson (1935), the investigation of necessity has centered its attention upon cell-wall constituents. In the edible portions of vegetables, the cell-membrane materials which are found most abundantly are cellulose, the hemicelluloses, and the pectic substances. Lignin is found to a considerable extent in some portions, but since this constituent is attacked by chemicals only with considerable difficulty, it need not be considered among the materials affected seriously by the mild conditions encountered in a cooking period.

Research workers interested in cell-membrane materials from widely different standpoints have traced metabolic changes in these constituents under various conditions. The softening of the tissues of fruits and vegetables during ripening and storage has been shown conclusively to involve changes in the pectic substances. In studying the pectic changes occurring during the ripening of apples, Dutt (1921) found that they exhibited a progressive increase in pectin, developed at the expense of pectose (protopectin); and these results were corroborated by Carré (1922) in more detailed chemical and microscopic studies of apples during different stages of growth.

Appleman and Conrad (1926) have made similar observations regarding the changes in the pectic substances of peaches as they ripen; and peaches have also been subjected to histological, macrochemical, and microchemical studies in this connection by Blake, Davidson, Addoms, and Nightingale (1930 and 1931). Problems of a "sloppy" pack in tomatoes led Appleman and Conrad (1927) to study the variation in content of the pectic substances in tomatoes; and problems in the rotting of potatoes during storage brought Dastur and Agnihorti (1934) to examine the changes in the pectic substances in potatoes during storage and rotting.

The results of all these studies have indicated that the alteration in the texture of fruits and vegetables during the ripening and rotting is, in part at least, a factor of the changes in the pectic substances. In every instance reported, soluble pectin is formed at the expense of protopectin up to a certain stage in the metabolic life of the plant (the stage when it is fully ripe). Beyond this point, decrease is found in pectin as well as in protopectin and, consequently, decrease is found in the total pectic substances. The intercellular substance maintains a fairly constant level throughout ripening, but as the overripe condition develops, this fraction decreases until there is a negligible amount of it in the tissue.

When these quantitative studies have been accompanied by microscopic examination, it has been found that the walls become progressively thinner during ripening and the intercellular substance disappears until finally the cells are found to be entirely separated from one another. Consideration of the conditions under which these changes proceed indicates that they are dependent upon the activity of enzymes present in the fruit and vegetable tissue.

It seemed interesting, then, to determine whether any relationship exists between the softening known to occur during the cooking of vegetables and changes in cell-membrane materials. Following is the report of such a study made by macrochemical, microchemical, and histological means.

QUANTITATIVE STUDY OF EFFECT OF STEAMING ON PECTIC SUBSTANCES OF CARROTS AND PARSNIPS

The preparation of vegetables and the method of steaming were planned to duplicate home methods as nearly as possible. The vegetables were pared, then cut lengthwise, and steamed in large steamers containing vigorously boiling water. Three similar lots were prepared, one lot was analyzed raw, one was steamed 20 minutes, and the last lot was steamed 45 minutes. Neither of these cooking periods represented the time at which the vegetable was just tender enough to serve. Since this was an original investigation in this field, there was no way of predicting whether the changes would be large or small and it was therefore deemed wise to include a period of cooking, which was rather extreme in duration. At the end of 20 minutes the vegetables were scarcely tender (particularly was this true of the parsnips), and at the end of 45 minutes they were both considerably overcooked, particularly the carrots. The results therefore represent changes during cooking rather than an analysis of either vegetable when suitable for serving.

Preparation of the Vegetable for Analysis

1. *Sampling the Vegetables:* Each carrot and parsnip was cut lengthwise in fourths through the center (since this can be accomplished readily) and one of the slices so obtained was used in each of the three lots for analysis; the remaining fourth slice was discarded. In each lot for analysis, approximately 600 grams of vegetable were used.

2. *Steaming the Vegetables:* So that the water collecting under the samples during steaming could be saved for analysis, the vegetable was put into a strainer, the strainer was placed over a bowl, and this whole arrangement was transferred to the steamer. One lot was steamed for 20 minutes and the other for 45 minutes.

3. *Slicing Into Boiling Alcohol:* Since plant tissues contain enzymes which are capable of changing protopectin to pectin and pectin to pectic acid, Norman (1937), it was imperative that such enzymes be inactivated as soon as possible after the vegetables were pared and cut. Treating the material with boiling alcohol is recommended by Conrad (1926) as the most efficient method of accomplishing this purpose.

Accordingly, the vegetables to be analyzed raw were sliced rapidly with a razor blade into boiling alcohol and boiled for 10 minutes. After boiling, the samples and alcohol were collected in large flasks and stored until time for drying. To permit enzyme action to proceed as in home cooking, the samples to be cooked were steamed without preliminary treatment with alcohol. It was necessary, however, to treat all samples similarly except for steaming, hence the steamed vegetables were treated with boiling alcohol as soon as they were cool enough to handle after steaming.

4. *Drying in a Vacuum Oven:* The alcohol was drained from the vegetables and the samples were dried to constant weight in a vacuum oven at 80°C.(176°F.), according to the recommendations of Link and Tottingham (1923).

5. *Grinding to a Fine Powder:* The dried samples were ground in an electric grinder until the entire portion would pass through a 60-mesh sieve.

Quantitative Extraction of Pectic Substances

The methods used for extraction of the pectic substances from the dried vegetables were modifications of those suggested by Nanji and Norman (1928).

1. *Pectin*: Five successive portions of boiling water (approximately 50 ml. each) were used for this extraction and the procedure was planned so that the total time of extraction was approximately 25 minutes. With the successive portions of boiling water the sample was ground in a mortar and then it was strained through bolting silk (Dufour No. 25, 200 mesh.) The combined extractions were brought to a boil to precipitate proteins and other materials interfering with the determination. Finally, the solution was cleared by filtering several times through a Büchner funnel, using hardened filter paper.

2. *Protopectin*: Since protopectin is the fraction of the pectic substances which is insoluble in water but is rendered soluble by treatment with dilute hydrochloric acid, it may be extracted from the residue after the removal of pectin, using dilute hydrochloric acid. For this purpose, Conrad (1926) found that extracting the ground vegetable for a period of one hour in thirtieth normal hydrochloric acid was the most efficient procedure.

In the present investigation an indirect method for determining protopectin was employed. A fresh sample of dried vegetable was boiled gently under a reflux condenser with thirtieth normal hydrochloric acid for 45 minutes. The mixture was then strained through bolting silk and the residue was refluxed as before with hydrochloric acid. The mixture was again strained through bolting silk and the filtrate was made neutral, using concentrated sodium hydroxide. It was then cleared by filtering several times through a Büchner funnel, using hardened filter paper.

Two such extractions removed all the pectin and protopectin simultaneously from the tissue. Hence, when the figure representing the pectin as previously determined was subtracted from the amount of pectic substance in this solution, the protopectin content was obtained.

3. *Pectic Acid and Pectates*: After extracting the pectin and protopectin, the only fraction of pectic substance remaining is the pectic acid or pectates and this fraction may be extracted by refluxing with dilute ammonium oxalate. This fraction was determined in the present investigation by difference; that is, from the figure representing the total pectic substance, the figure for the sum of the pectin and protopectin was subtracted, the difference representing the amount of pectic acid or pectates present.

4. *Total Pectic Substance*: For this extraction a fresh sample of dried vegetable was refluxed for 30 minutes with thirtieth normal hydrochloric acid, then the solution was strained through bolting silk and the filtrate was made neutral. The residue was refluxed

for 30 minutes with .1 per cent ammonium oxalate and strained as before. This procedure of two extractions (one with hydrochloric acid and one with ammonium oxalate) was repeated and the pectic substance was then found to be completely removed from the tissue. The combined solutions were made neutral and they were then cleared by filtering several times through a Büchner funnel, using hardened filter paper.

Quantitative Determination of Pectin in Extracted Solutions

Emmet and Carré (1926) devised a combination of the alcohol and calcium pectate methods for determination of pectin, and a modification of their method was used in the present investigation.

A solution of pectin was added to acidified ethyl alcohol and allowed to stand several hours. The solution was then filtered through filter paper and the colloidal precipitate was washed with acidified alcohol. Boiling water was added to the precipitate on the filter paper to dissolve the pectin. The pectin solution passed through the filter paper and was collected in a beaker.

To the solution of pectin so obtained, tenth normal sodium hydroxide was added and the solution was allowed to stand at least several hours. Then normal acetic acid was added and after five minutes, a molar solution of calcium chloride was added to precipitate calcium pectate. To insure complete precipitation the solution was allowed to stand for at least several hours.

This solution containing calcium pectate was boiled five minutes to dissolve as much as possible of the chlorides, then it was filtered through hardened paper in a Büchner funnel. The precipitate was washed with boiling water until it was free from chlorides, then transferred to a small weighing bottle previously dried to constant weight. The calcium pectate was dried to constant weight at 100°C. (212°F.).

On each extraction solution, five determinations of pectin were made by this method, and the following is a typical set of five weights of calcium pectate so obtained:

.0227 gm. calcium pectate
.0230 gm. calcium pectate
.0220 gm. calcium pectate
.0213 gm. calcium pectate
.0224 gm. calcium pectate.

Consideration of the results from carrots (Table 1) makes it evident that a change has taken place in the ratio of the various pectic substances during steaming. The amount of pectin has increased more than 50 per cent during 20 minutes of steaming (from scarcely

four per cent to almost six per cent) and by the end of 45 minutes it has more than doubled the amount originally present (an increase of from scarcely four per cent to more than eight per cent). On the other hand, the amount of protopectin has decreased, and at the end of the 45-minute period only one-fourth (approximately) of the original amount of protopectin remains (a decrease of from 14 per cent in the raw sample to 3.6 per cent in the sample steamed for 45 minutes). Obviously, these changes have been brought about by the hydrolysis of protopectin to form pectin. If this were the only change occurring, however, the increase in pectin would be exactly the same as the decrease in protopectin and this is not the case. The decrease in protopectin is considerably greater than the increase in

TABLE 1
*Effect of Steaming on Pectic Substances of Carrots*¹

Fraction of pectic substance	Sample of carrot		
	Raw	Steamed 20 minutes	Steamed 45 minutes
Pectin.....	3.7	6.0	8.8
In cooking water.....	0.0	0.1	0.5
In vegetable.....	3.7	5.9	8.3
Protopectin (indirect method).....	14.1	9.1	3.6
Pectic acid (or pectates).....	0.8	1.0	1.3
Total pectic substance.....	18.6	16.1	13.7
In cooking water.....	0.0	0.1	0.5
In vegetable.....	18.6	16.0	13.2

¹ Results represent the per cent of dry weight and are computed from the average of five weights of calcium pectate in each case.

pectin and it may therefore be concluded that some of the pectin itself has been decomposed during steaming. This conclusion is verified by a decrease in the total pectic substances (from 18.6 per cent in the raw sample to 13 per cent in the sample steamed 45 minutes). A slight increase is found in the pectic acid or pectates of the vegetable.

Changes in the pectic substances of parsnips (Table 2) are in the same direction. The pectin and pectic acid (or pectates) have increased and the protopectin and total pectic substances have decreased. The changes in parsnips are slightly less than those in carrots, however, and this finding is significant in view of the fact that at the end of the steaming period the parsnips were not quite as soft as the carrots.

It has thus been established that progressive changes occur in the pectic substances of carrots and parsnips during cooking, and these changes are somewhat greater in carrots than in parsnips in

a given time. In every instance the changes are in the direction of an increase in pectin and in pectic acid (or pectates) and a decrease in protopectin and in the total pectic substances. The changes are similar to those which occur during the ripening of fruit and vegetable tissue and in both instances they are no doubt related to the softening which takes place.

HISTOLOGICAL STUDY OF EFFECT OF STEAMING ON PECTIC SUBSTANCES AND CELLULOSE OF CARROTS AND PARSNIPS

For the purpose of observing structural changes which take place in carrots and parsnips during cooking, it was necessary to prepare

TABLE 2
*Effect of Steaming on Pectic Substances of Parsnips*¹

Fraction of pectic substance	Sample of parsnip		
	Raw	Steamed 20 minutes	Steamed 45 minutes
Pectin.....	4.7	6.1	7.9
In cooking water.....	0.0	0.1	0.7
In vegetable.....	4.7	6.0	7.2
Protopectin (indirect method).....	10.2	7.7	5.7
Pectic acid (or pectates).....	1.6	2.0	2.1
Total pectic substances.....	16.4	15.8	15.7
In cooking water.....	0.0	0.1	0.7
In vegetable.....	16.4	15.7	15.0

¹ Results represent the per cent of dry weight and are computed from the average of five weights of calcium pectate in each case.

paraffin sections of these vegetables. With a view to determining the extent to which such changes are progressive during the steaming period, 15-minute periods of steaming were chosen. For both vegetables, one lot was left raw, one was steamed 15 minutes, one 30 minutes, and the last lot was steamed 45 minutes.

Vegetables were pared as for home cooking, then samples were cut in such a variety of ways that transverse, radial, and tangential sections were provided from the stem end, the central part, and the tip end of the roots.

Immediately after cutting, the samples to be studied raw were placed in vials with Nawaschin's fluid, and those to be steamed were placed in the steamer for the required length of time. When cool, the steamed samples were placed in vials filled with Nawaschin's fluid. After standing for 36 hours in this fluid, all the samples were washed in running tepid water and dehydration was accomplished by running the specimens through a series of alcohol solutions of gradually increasing strength. Finally, they were embedded in paraffin.

Sections from 10 to 12 microns thick were cut with the rotary microtome and, using sections so obtained from carrots and parsnips, the following studies were made:

Cellulose: For studying the cellulose the sections were stained with light green, using a one-per cent solution in 90-per cent alcohol, Conn (1929). This stain is by no means specific for cellulose, but in all probability a large part of the material staining green with it in these specimens was cellulose.

A decided difference was found between the cellulose of the raw samples and that of the steamed ones. In the raw samples a large proportion of the cell walls was relatively thick and continuous, whereas in the steamed ones the walls were thin and broken. This

TABLE 3
Effect of Steaming on Pectic Substances of Carrots and Parsnips¹

Fraction of pectic substance	Raw		Steamed 20 minutes		Steamed 45 minutes	
	Carrots	Parsnips	Carrots	Parsnips	Carrots	Parsnips
Pectin.....	3.7	4.7	6.0	6.1	8.8	7.9
Protopectin.....	14.1	10.2	9.0	7.7	3.6	5.7
Pectic acid or pectates.....	0.8	1.6	1.0	2.0	1.3	2.1
Total pectic substance.....	18.6	16.4	16.1	15.8	13.7	15.7

¹ Results represent the per cent of dry weight and are computed from the average of five weights of calcium pectate in each case.

change was progressive during the steaming period and was found in all sections from each portion of both vegetables.

In the raw samples, regardless of the portion of the root to be examined, the cell walls of the xylem tissues were considerably thicker and more continuous than those of either the cambium or the phloem. A corresponding difference was observed between the cell walls of the xylem tissue and other tissues of the steamed samples. That is, in the samples steamed 45 minutes a considerable proportion of the cell walls of the xylem was found to have a clear though faint outline, whereas the cell walls of the cambium and phloem were very thin and incomplete indeed. This observation is significant in view of the fact that it requires slightly longer for the inner (xylem) portion of the root to become tender during cooking than for the outer (phloem) portion.

These same general results were found with both carrots and parsnips. The results on the whole, however, were slightly more extreme from carrots than from parsnips and this finding is in accordance with the fact that the same time of steaming produces slightly greater softening in carrots than in parsnips.

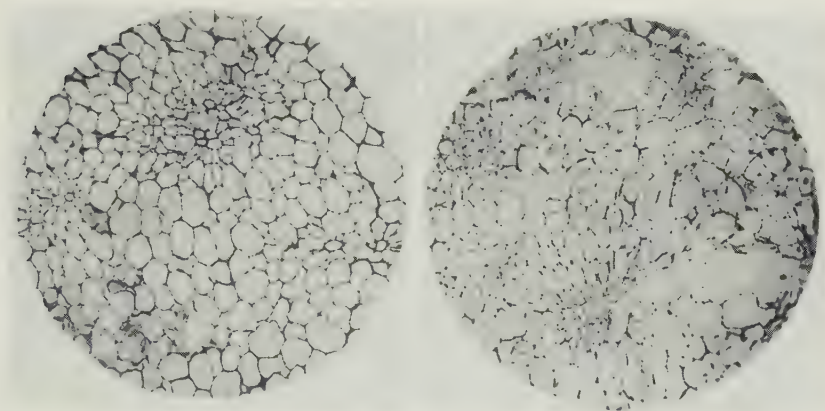
PLATE I

EFFECT OF STEAMING ON THE PECTIC SUBSTANCES
OF CARROTS

Transverse sections

Magnification approximately $\times 50$

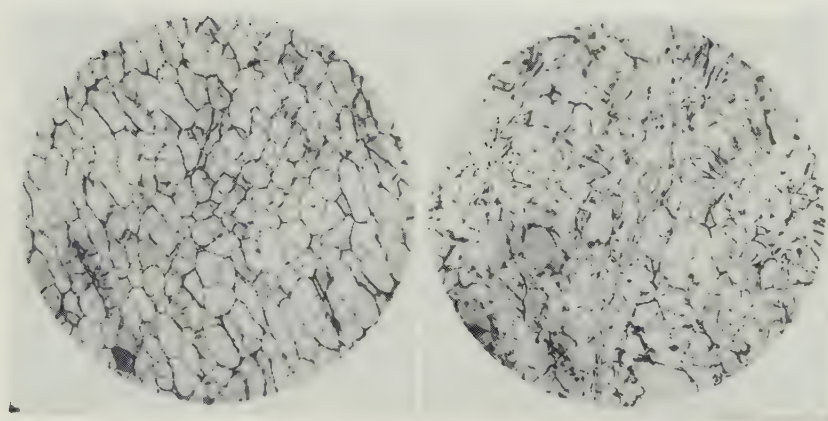
XYLEM TISSUE



Raw

Steamed 45 minutes

PHLOEM TISSUE



Raw

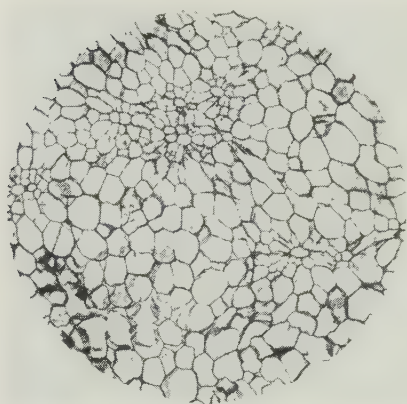
Steamed 45 minutes

PLATE 2
EFFECT OF STEAMING ON THE CELLULOSE OF CARROTS

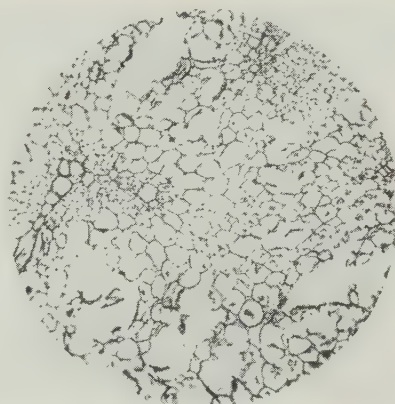
Transverse sections

Magnification approximately $\times 50$

XYLEM TISSUE

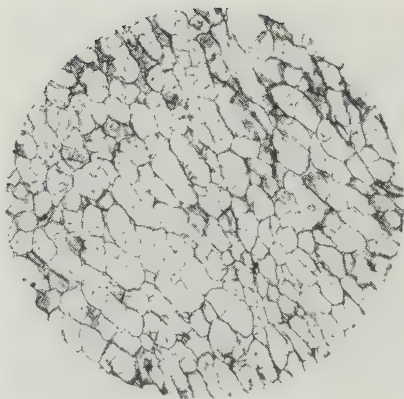


Raw

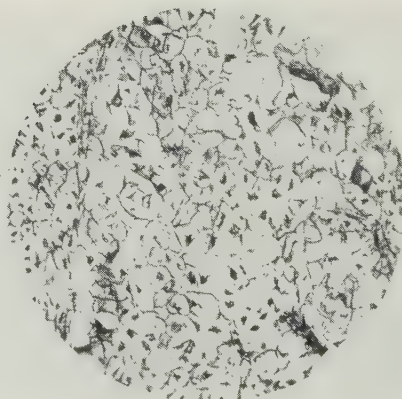


Steamed 45 minutes

PHLOEM TISSUE



Raw



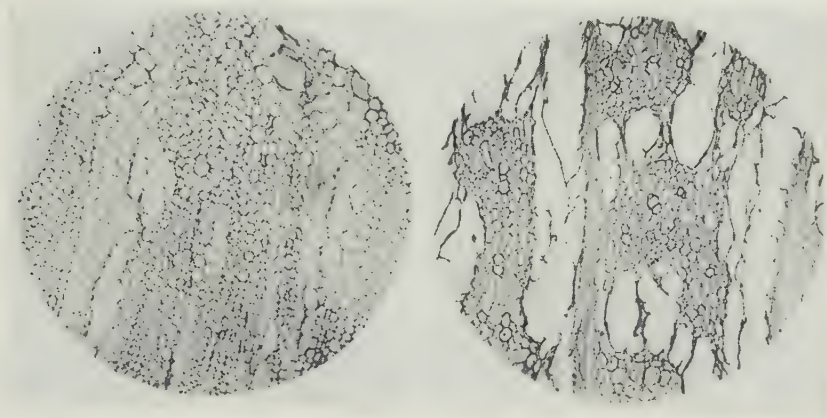
Steamed 45 minutes

PLATE 3
EFFECT OF STEAMING ON THE PECTIC SUBSTANCES
OF PARSNIPS

Transverse sections

Magnification approximately $\times 50$

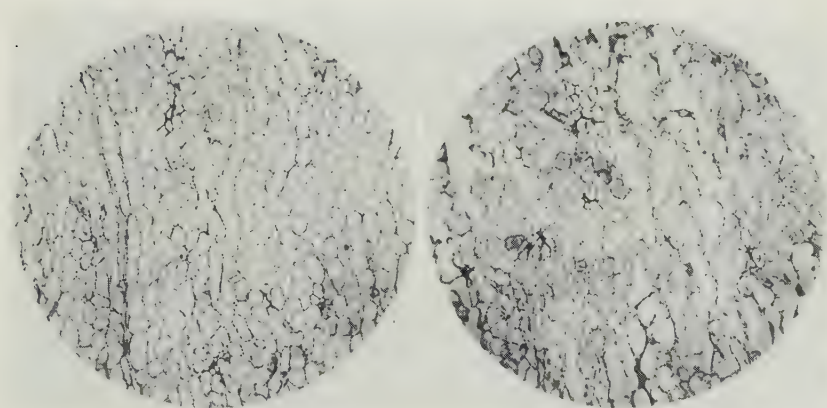
XYLEM TISSUE



Raw

Steamed 45 minutes

PHLOEM TISSUE



Raw

Steamed 45 minutes

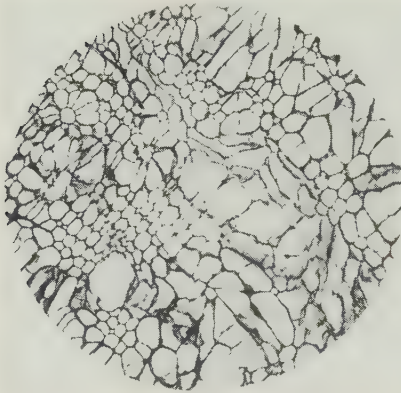
PLATE 4

EFFECT OF STEAMING ON THE CELLULOSE OF PARSNIPS

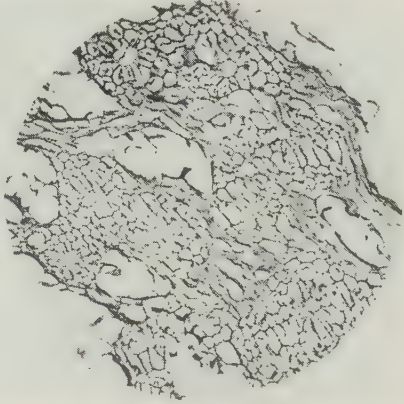
Transverse sections

Magnification approximately $\times 50$

XYLEM TISSUE



Raw



Steamed 45 minutes

PHILOEM TISSUE



Raw



Steamed 45 minutes

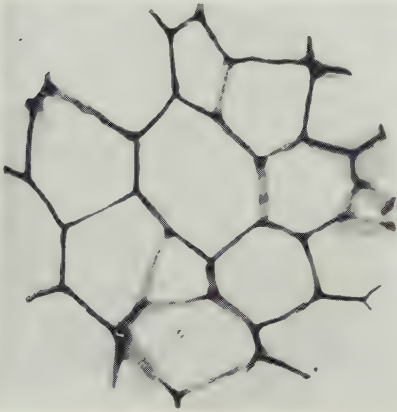
PLATE 5

ENLARGEMENTS SHOWING DETAILS OF THE EFFECT OF
STEAMING ON THE XYLEM TISSUE OF CARROTS

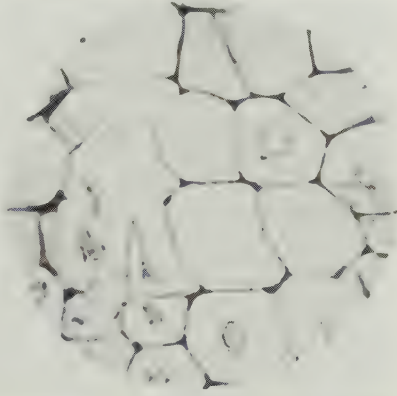
Transverse sections

Magnification approximately $\times 200$

PECTIC SUBSTANCES

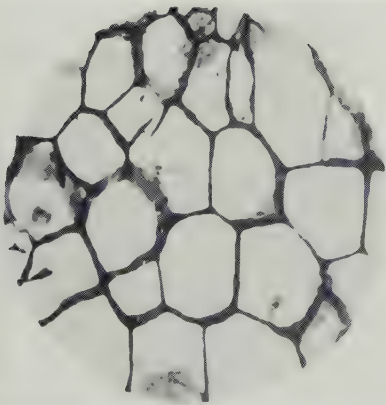


Raw

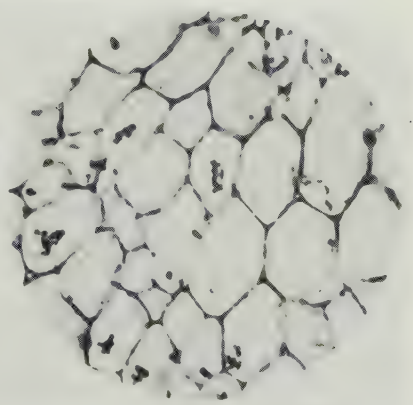


Steamed 45 minutes

CELLULOSE



Raw



Steamed 45 minutes

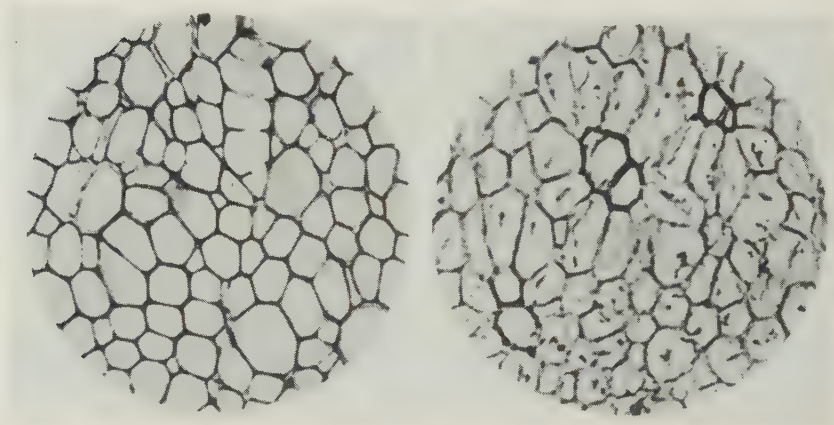
PLATE 6

ENLARGEMENTS SHOWING DETAILS OF THE EFFECT OF
STEAMING ON THE XYLEM TISSUE OF PARSNIPS

Transverse sections

Magnification approximately $\times 200$

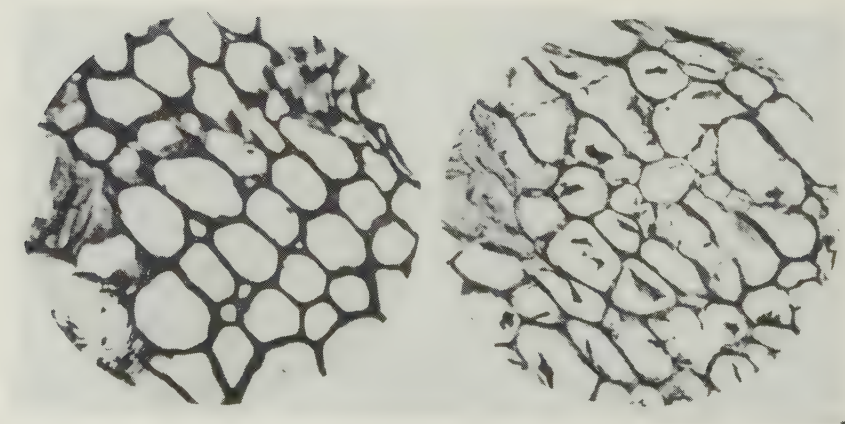
PECTIC SUBSTANCES



Raw

Steamed 45 minutes

CELLULOSE



Raw

Steamed 45 minutes

Pectic Substances: The stain used for the pectic substances was ruthenium red (one part ruthenium red in 10,000 parts distilled water). This stain is not specific for the pectic substances but no doubt a large part of the material staining with it in these samples (proved by quantitative chemical analysis to have considerable pectic material) was pectic in nature.

A study of the sections showed that the alterations during steaming were very similar to those already noted in the samples stained to show cellulose.

These observed differences between the pectic substances of the raw and steamed samples may no doubt be attributed to gradual hydrolysis of the protopectin (insoluble) to pectin (soluble) during the steaming period. The preparation of the sections involved extensive washing with water and hence any soluble pectin present was inevitably washed away from them. Therefore, the difference in the amount of pectic substances between the raw and steamed samples as noted in these sections represented the amount of protopectin hydrolyzed during steaming. Careful examination of the cell walls revealed the fact that the loss of pectic substance was particularly evident in the regions of the primary wall and the intercellular areas.

A relationship has thus been established between the condition of the structural framework of these vegetables and the gradual loss of rigidity which they suffer during cooking. For making a permanent record of these findings, photomicrographs were taken of representative transverse sections of both xylem and phloem tissues of the raw vegetables and also of those steamed for 45 minutes. Enlargements were made from the xylem tissue of the samples to record in greater detail the changes in the cell walls during cooking. These photomicrographs and enlargements are to be found in Plates 1, 2, 3, 4, 5, and 6.

MICROCHEMICAL STUDY OF EFFECT OF STEAMING ON CELLULOSE OF CARROTS AND PARSNIPS

Behavior With Iodine in Potassium and Sulphuric Acid: When cellulose is subjected to the action of rather strong sulphuric acid, a substance is formed which gives a blue absorption compound with iodine. The substance so formed is hydrocellulose or amyloid (a polysaccharide similar to starch in that it gives a blue color with iodine).

A solution of iodine in potassium iodide was allowed to remain on a series of paraffin sections of raw and steamed carrots and parsnips for one-half hour, then the sections were washed with distilled water and a drop or two of approximately 70 per cent sulphuric

acid was added. It was observed that the blue color and swelling owing to the adsorption compound which is formed between hydrocellulose and iodine appeared very rapidly in the steamed samples and very slowly in the raw ones. Such a difference indicates that steaming has altered the cellulose in some manner, making it more readily affected by the reagent. It may be that the cellulose has been liberated to some extent from its surrounding materials in the cell wall, making it more accessible to the reagent; or since the reaction by which hydrocellulose is formed is one of hydrolysis, it seems probable that hydrolysis of the cellulose in the steamed samples has already begun, and it is therefore more readily acted upon by the reagent. This reaction involves considerable swelling of the walls, hence it cannot be used for studying the structure of the cell walls of the tissue.

Behavior With Chlor-Zinc-Iodide: This reagent also hydrolyzes cellulose to hydrocellulose (or amyloid) but it does not cause the cell walls to swell to the extent that they do when treated with sulphuric acid and iodine in potassium iodide, and it can therefore be used not only as a test for cellulose but also for studying the structure of the cell walls.

Paraffin sections from the raw and steamed carrots and parsnips were flooded with chlor-zinc-iodide and allowed to stand. In the carrot and parsnip samples steamed for 45 minutes, the blue-violet color appeared almost immediately. After standing for 45 minutes, a definite gradation in intensity was obtained from pale violet in the raw samples, deepening with successive periods of steaming to a decided blue-violet color in those steamed for 45 minutes. Evidence was thus again obtained for a progressive change in the cellulose with increase in the time of steaming. Furthermore, the effect of steaming on the cell walls, as observed by their appearance when stained with light green, was confirmed by their appearance when treated with chlor-zinc-iodide, namely that the cell walls showed progressive loss of thickness and continuity during the steaming period.

SUMMARY

The purpose of this study was to observe some of the changes which take place when vegetables are cooked and which are in all probability related to the softening known to occur. Since the rigidity of plant tissues is dependent upon their cell-wall constituents, the problem has necessarily centered upon the cell-membrane materials. Using carrots and parsnips, chemical, histological, and microchemical studies have been made to observe the manner in which the pectic substances and cellulose are affected by cooking.

Chemical Study: Three samples of both vegetables were prepared, one of them was left raw, one was steamed 20 minutes, and the last was steamed 45 minutes. The samples were treated with boiling alcohol to stop enzyme action, then dried in a vacuum oven at 80°C. (176°F.), and ground to a fine powder. On each dried sample, determinations were made of the pectin, protopectin, pectic acid (or pectates), and total pectic substances. The content of pectin was found to have increased progressively during steaming, and the protopectin to have decreased in both vegetables. A slight increase was found in the pectic acid (or pectates) and a slight decrease in the total pectic substances of the samples. The changes throughout were slightly greater in carrots than in parsnips.

Histological Study: A series of paraffin sections of both raw and steamed vegetables was stained with light green to show cellulose, and another series was stained with ruthenium red to show the pectic substances. In all cases the cell walls of the steamed samples were less thick and less continuous than those of the raw ones. Such disintegration is no doubt an important factor in the loss of rigidity of the vegetables during steaming. Photomicrographs were taken of representative sections of xylem and phloem tissue of the raw vegetables and of those steamed 45 minutes. Enlargements were made from the xylem tissue of these samples.

Microchemical Study: Using paraffin sections of both vegetables, microchemical tests were made of the cellulose in the samples, using iodine in potassium iodide and sulphuric acid, also using chlor-zinc-iodide. These tests proved that a decided difference existed between the cellulose of the raw samples and that of the steamed ones, such changes occurring progressively throughout the steaming period.

Evidence has thus been obtained by chemical, histological, and microchemical means of changes which occur in cell-membrane materials of carrots and parsnips during cooking. The alterations that have been observed indicate a relationship between the disintegration of the tissue and the change in texture of the vegetable when it is cooked.

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